Response of broilers subjected to an enteric challenge and fed diets with varying limestone particle sizes and calcium concentrations–part 1: performance, tibia mineralization, and nutrient digestibility[1]

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ABSTRACT

This 35-d experiment evaluated the effects of limestone particle size and calcium concentrations on broiler performance, tibia mineralization, and nutrient digestibility. Two thousand one hundred d-old YPM x 708 male broilers were distributed into 70 floor pens and assigned to 1 of 7 treatments (10 replicates/treatment). This experiment was a 2 x 3 + 1 factorial arrangement, including 2 limestone particle sizes (910 and 200 µm) and 3 calcium concentrations (adequate, reduced, and low), and an unchallenged control. Dietary calcium was a two-step, 10-point reduction from adequate concentrations (i.e., breeder recommendations). All factorial treatments were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*. Performance (d 17, 21, 26, and 35), tibia mineralization (d 21 and 35), and nutrient and energy digestibility (d 21 and 35) were measured. The enteric challenge reduced BW and increased feed conversion ratios (**FCR**; contrast, $P \leq 0.05$). Broilers fed adequate and reduced calcium diets had higher BW, tibia shear strength, and tibia ash on d 35 compared to low calcium diets (main effect, $P \leq 0.05$). Day 1 to 35 FCR linearly increased as dietary calcium decreased from adequate to low in the group fed 910 µm limestone, whereas in the 200 µm limestone group, only the low calcium and not the reduced calcium led to higher FCR compared with those fed adequate calcium (interaction, $P \leq 0.05$). Broilers fed reduced and low calcium diets with 910 µm limestone had the highest calcium and phosphorus digestibility on d 21. With 200 µm limestone, broilers fed a low calcium diet had higher mineral digestibility (interaction, $P \leq 0.05$). Day 35 apparent ileal digestible energy (**AIDE**) increased when broilers consumed an adequate or reduced calcium diet compared to low calcium (main effect, $P \leq 0.05$). This experiment demonstrated dietary calcium concentrations 0.10 percentage units lower than recommended concentrations can maintain broiler performance, tibia

mineralization, and AIDE during an enteric challenge. However, calcium concentration effects depended on limestone particle size.

Key words: limestone particle size, calcium, *Eimeria*, *Clostridium perfringens*, broiler

INTRODUCTION

Antibacterial growth promotors (**AGP**) have been used to improve poultry production efficiency, weight gain, and feed conversion (Miles et al., 2006; Maria Cardinal et al., 2019). However, AGP usage has declined in recent years, which may result in reduced broiler performance and higher incidence of diseases such as coccidiosis and necrotic enteritis (**NE**) (Engster et al., 2002; Cervantes, 2015). Necrotic enteritis is a multifactorial enteric disease caused by *Clostridium perfringens*, particularly after birds have been predisposed by *Eimeria* infection (Emami and Dalloul, 2021). Various nutritional strategies aimed at improving broiler performance and health without AGP have been reviewed to mitigate the severity of these enteric diseases (Dahiya et al., 2006; Ayalew et al., 2022). These nutritional strategies primarily rely on feed additives (e.g., organic acids and probiotics), however, controlling dietary calcium concentrations and particle size of the calcium source could be a viable strategy to mitigate enteric disease severity.

Calcium is an important macro mineral in broiler nutrition, serving as a structural component of bones, intracellular messenger, and cofactor of various enzymes (Proszkowiec-Weglarz and Angel, 2013). While adequate calcium is essential for normal development and physiological processes, broiler diets containing high calcium concentrations or highly soluble calcium sources could negatively impact growth and health, particularly under NE challenge conditions (Paiva et al., 2013; Paiva et al., 2014). Due to the low cost and widespread availability of commonly used calcium sources (David et al., 2023), excessive inclusion of calcium in broiler diets is common, emphasizing the need for stricter control over its concentration (Walk, 2016; Li et al., 2017).

High calcium concentrations may contribute to NE pathogenicity through multiple mechanisms. It can promote the synthesis and activity of NetB and α-toxin produced by *C. perfringens*, which disrupts the intestinal mucosa (Keyburn et al., 2010; Fathima et al., 2022). Furthermore, high calcium concentrations can increase calcium-phytate complex formation (Selle et al., 2009), resulting in undigested nutrients that can serve as substrates for *C. perfringens* (Moran, 2014). Beyond dietary calcium concentration, solubility of the calcium source may also influence NE severity (Paiva et al., 2013).

Limestone is a commonly used calcium source in broiler diets worldwide, but its characteristics vary significantly across regions (Gilani et al., 2022). Variation in limestone characteristics such as particle size can influence its solubility, with smaller particles resulting in higher *in vitro* solubility (de Witt et al., 2006; Kim et al., 2019), but a lower *in vivo* solubility (de Witt et al., 2006). When comparing particulate (402 μm) and pulverized limestone (< 75 μm), Kim et al. (2018) reported that pulverized limestone negatively impacted apparent ileal digestibility (**AID**) of calcium and phosphorus (**P**). Furthermore, Paiva et al. (2013 and 2014) reported a negative impact of higher dietary calcium concentrations and calcium solubility on broiler growth during a naturally occurring clinical NE challenge. Zanu et al. (2020a) expanded on this by examining dietary calcium and phytase concentrations during a subclinical NE challenge, finding that higher dietary calcium combined with the NE challenge reduced CP, carbon, potassium, and zinc digestibility. However, there is still limited data on the response of broilers fed varying limestone particle size and calcium concentrations under subclinical NE conditions. Therefore, the objective of the current experiment was to challenge broilers with *Eimeria* spp. and *C. perfringens* and evaluate the interactive effects of limestone particle size and calcium concentrations on performance, tibia mineralization, and nutrient digestibility.